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TECHNIQUES FOR DETERMINING TOTAL BODY WATER USING
DEUTERIUM OXIDE

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ABSTRACT

The measurement of total body water (TBW) is fundamental to the study of body fluid changes consequent to microgravity exposure or treatment with microgravity countermeasures. Often, the use of radioactive isotopes is prohibited for safety or other reasons. It was desired that a safe method of total body water measurement be selected and implemented for use by some Johnson Space Center (JSC) laboratories, which permitted serial measurements over a 14 day period which was accurate enough to serve as a criterion method for validating new techniques. These requirements resulted in the selection of deuterium oxide dilution as the method of choice for TBW measurement. This report reviews the development of this technique at JSC. The recommended dosage, body fluid sampling techniques, and deuterium assay options are described.

INTRODUCTION

The measurement of total body water (TBW) is necessary for studying the response of body fluids to microgravity and microgravity countermeasures. The measurement of TBW with deuterium oxide (D₂O), has been well studied. This method is safe, non-radioactive, and potentially very accurate, and is the method of measuring body water turnover in doubly-labeled water studies of long-term metabolic rate (Schoeller et al., 1980; Wong et al., 1988; Schoeller, 1990). By using low doses and measuring the baseline D₂O level, repeated TBW measurements can be made over several days without compromising subject safety.

DOSAGE

A wide range of D₂O dosages have been used in TBW determinations. The chief limiting factor in determining minimal dosage is the precision of determination of D₂O diluted in a body fluid sample. Consequently, minimal dosage is a function of the volume of TBW and the precision of the D₂O assay. Minimal dosage reported in the literature for adults was 1 g (Schoeller et al., 1980). Such a dosage results in a D₂O concentration increase of 29 ppm in a 60 kg female at 20% fat with assumed water fraction of 73% of the fat free mass or 24 ppm in a 70 kg male with 15% fat. The largest D₂O dosage reported in the literature was 107 g for a 70 kg body weight male (Nielsen et al., 1971). The natural abundance of D₂O in tap water varies, but is approximately 140-150 ppm (Thomson, 1963; Davis et al., 1987). The body tends to concentrate D₂O slightly, yielding saliva baseline concentrations of about 0.02-0.03 ppm above local levels of ingested water (Halliday and Miller, 1977). This suggests that a 1 g dose of 99.9% D₂O would raise the baseline value only about 16% for males.

Because many NASA applications require repeated TBW measurements over 10-13 days, the baseline concentration of D₂O will increase based upon an elimination half-life of about 10 days (Schloerb, et al., 1950; Schoeller et al., 1980; Schoeller and Webb, 1984; Wong, et al., 1988; Schoeller, 1990). For maximum accuracy, it is necessary to minimize baseline levels and maximize D₂O concentration differences between baseline and post dose. This is accomplished by keeping each dose as small as can be accurately

measured after dilution in body fluids. For the purposes of a 13 day bedrest with requirements to determine TBW before and after a lower body negative pressure and fluid-loading countermeasure, the following doses were administered on the days shown:

		<u>Estimated D2O Conc. (ppm)*</u>			
Day	Dose (grams)	Pre	Dose	Post	% Increase
- 1	4	150	114	264	76
5	4	218	114	332	52
6	6	317	171	488	54
10	6	349	171	520	49
11	7	438	228	638	46

* Estimated concentrations for 60 kg subject with 20% fat and 73% of fat free mass as water.

Total doses=5; total dosage= 27 g.

In cooperation with Dr. Suzanne Fortney of the JSC Cardiovascular Research Laboratory, this scheduled dosage was administered to one bed rest subject and six controls. This work is currently in progress.

Risks

Toxicity of D2O to humans has not been precisely determined, but it has been used in human research for over 30 years without reported ill effects. The TD50 required for reproductive effects in animals is 840 g/kg weight (Registry of Toxic Effects, 1986). Animal studies show that D2O concentration must reach 30-35% of total body water to be lethal. Fusch and Moeller (1988) suggest that D2O concentrations for short term studies be less than 10 g/ kg of body water. Schloerb et al. (1950) found no effects in healthy or ill subjects receiving D2O doses of 100g. Doses in this range far exceed that normally required for TBW determinations using sensitive deuterium assay methods.

D2O has been reported to produce nystagmus (Money and Miles, 1974). In recent studies at Johnson Space Center, a dose of approximately 200 g for a 70 kg subject was required to produce symptoms of vestibular impairment in . Therefore, the maximal total exposure of 28 g is extremely safe.

FLUID SAMPLING

One of the advantages of D2O measurement of TBW, is that serum, urine, respiratory water, and saliva have all been used successfully in the technique. For convenience, safety, and requirements for non-invasive methods for space flight, saliva sampling is reviewed as a recommended fluid sampling technique. The D2O method requires an initial fluid sample to determine baseline D2O levels. This can be collected by having the subject expectorate into a small vial. Only approximately 2 ml of saliva is needed, but a capped 5-10 ml vial such as the Sarstedt saliva collection kit used at JSC is convenient to use. It is important that the subject refrain from eating or drinking for 2 hours prior to saliva sampling as the potential exists for obtaining saliva diluted with ingested fluid. Food or drink ingested immediately prior to or following D2O dosing may retard equilibration of D2O with body water. D2O doses should be weighed to the nearest milligram or better in order to maintain overall precision in the ppm range (Schoeller et al., 1980). Because the doses are small, care must be taken to ensure the subject does not lose any of the deuterium. This is accomplished by rinsing the dose vial with at least half its volume of deionized water and having the subject ingest the rinse water. This rinsing is done twice (Thomson, 1963). Equilibration has been found to take about three hours in resting subjects (Schloerb, et al., 1950; Schoeller et al., 1980; Lukaski and Johnson, 1985; Wong et al., 1988). During the equilibration period, no food or drink should be taken. Since any D2O lost must be accounted for in TBW determinations, urine voids during this time must be sampled for D2O concentration and the volume recorded to adjust for this loss. Halliday and Miller (1977) have reported that fractionation results in unequal distributions of D2O in the baseline samples with urine, plasma, serum, and saliva, all showing concentrations higher than local drinking water and respiratory water vapor showing lower baseline concentrations. Fractionation occurs because the heat of vaporization is 3%, and the heat of fusion is 5.5%, higher in D2O compared to H2O (Thomson, 1963). Hence, baseline and equilibration samples should be drawn from the same type of fluid.

DEUTERIUM ASSAY

Methods for deuterium assay include, infrared spectrophotometry, gas chromatography, and radio-isotope ratio mass spectroscopy (Graystone, et al., 1967; Thomson, 1963). Other methods include freezing point depression,(Reaser and Burch, 1958), near-infrared spectrophotometry, refractometry, and falling-drop, (Thomson, 1963); however, these methods are not sufficiently accurate for the dilutions used in our applications and will not be discussed.

Infrared spectrophotometry

Infrared spectrophotometry has been used extensively in assaying D₂O. It requires careful and laborious sample preparation. Lukaski and Johnson (1985) tested the recovery of plasma and urine using several treatment protocols. Chemical precipitation of proteins with copper sulfate, cadmium chloride, ferrous chloride, mercuric chloride, and stannous chloride and treatment with activated charcoal all yielded either turbid supernatants, or supernatants could not be recovered. Only vacuum sublimation or distillation yielded high recoveries. Turner et al. (1960) and Fusch and Moeller (1988) used vacuum distillation in which liquid samples were processed at pressures of 0.05 to 1 mmHg with the vapor subsequently refrozen in liquid nitrogen or dry ice and isopropanol. Stansell and Mojica, (1968) used distillation (under atmospheric pressure) with condensate collected in a water-cooled tube.

Davis et al. (1987) used diffusion in Conway dishes to obtain clean samples. The diffusion technique is simpler than most other sample cleansing methods, but it may be inaccurate due to fractionation between D₂O and H₂O (Wong and Klein, 1986). Also accuracy diminishes because the actual concentrations measured are halved in this method. Volumetric errors in either the sample or the H₂O are possible, and to be exact, the saliva water volume (rather than saliva volume) should be matched to the H₂O volume. Additionally, the diffusion method would be very laborious for a large number of samples.

For potential use in JSC laboratories, a vacuum distillation system was constructed and tested. Approximately 2 ml of unpurified sample was placed in a stoppered sample tube connected with a small plastic three-way valve to a 7 ml vacutainer with two-inch pieces of tygon tubing. Both the sample tube and the vacutainer were initially heated to approximately 45°C in a water bath. Vacuum was then applied to both tubes via the three-way valve with a Dayton (1/4hp) vacuum pump. The stopcock was then turned to seal off the vacuum side and maintain a vacuum in the two-tube system. The sample tube and vacutainer were then allowed to cool to room temperature before the vacutainer was placed in an acetone-dry ice mixture. Failure to allow the sample to cool before chilling the vacutainer lowered the system pressure too low, resulting in boil-over of the sample which contaminated the vacutainer. After a few minutes, water vapor evolving from the sample raised the pressure sufficiently to allow returning the sample to the water bath. The sample then distilled over several hours until the sample was reduced to dryness. It is important that the sample be dried because D₂O fractionates at a higher temperature than H₂O resulting in an artificially low D₂O concentration if vaporization is incomplete (Thomson, 1963).

Spectroscopic analysis of the O-D vibrational band at 2500 cm⁻¹ (3.98 nm) is conducted with either a single beam fixed-filter (Lukaski and Johnson, 1985; Stansell and Mojica, 1968) or a dual beam (Turner et al., 1960) spectrophotometer in a calcium fluoride cell Thermostated at 15 or 20 °C (Lukaski and Johnson, 1985) or 30 and 48 °C (Stansell and Mojica, 1968) for sample and reference cells, respectively. For single beam machines, deionized water is used as the zero reference. Between samples, some investigators cleaned the cell with dry nitrogen, (Lukaski and Johnson, 1985) whereas others did not (Stansell and Mojica, 1968). Davis et al. (1987) were able to measure down to 30 ppm. Reported precision of the single beam method is 2.5% (Lukaski and Johnson, 1985).

Gas chromatography

The gas chromatography (GC) analysis for D₂O requires minimal sample preparation. A 50 microliter sample is injected into an evacuated column containing calcium hydride (Arnet and

Duggleby, 1963; Wong and Klein, 1986). Protium and deuterium gas evolves and is injected through a 7 or 8 port valve, into a chromatograph equipped with a 5-20 ml sample loop. The gas is then passed through a 1 meter long activated charcoal column held at room temperature, where the hydrogen is cleaned further. From the column, the gas passes to the detector where the difference in thermal conductivity between the deuterium enriched gas from the sample, and the hydrogen carrier gas is measured with a thermal conductivity cell thermostated at 100°C (Arnet and Duggleby, 1963). The thermal detector cell voltage is output to a recorder. Any deuterium in the carrier gas is zeroed out during set-up. The peak height is measured or better, the area under the curve is integrated to measure concentration relative to known standards.

Mendez et al., (1970) examined the methodology of GC analysis and found it to be as accurate as infrared analysis at a concentration of 1085 ppm (0.12% w/v). They also examined vacuum sublimation and found it did not significantly improve the accuracy or precision of the analysis. In fact, both untreated and vacuum distilled saliva produced exactly the same mean and standard deviation for 11 samples at a concentration of 1356 ppm (0.15%). Duplicate samples showed very high test-retest means, differing by 1 ppm at 1175 ppm (0.13%). Mean recovery in urine samples was 99.3%.

Ratio-isotope mass spectroscopy

Ratio-isotope mass spectroscopy is the most common method of measuring deuterium concentrations in studies which use doubly-labeled water to measure metabolic rate. D₂O is assayed because the doubly labelled water technique requires that O₁₈ respiratory turnover be adjusted for water losses of O₁₈. In this method, the fluid sample is treated with a hot reactive metal such as uranium or zinc, thereby liberating gaseous hydrogen. The deuterium and protium evolved are then measured on a mass spectrometer configured for deuterium/protium analysis. This method can measure very low concentrations of deuterium. The chief disadvantages of this technique is the very intricate labware required for sample preparation and the expense of the mass spectrometer. Currently the Planetary Sciences Division of JSC is setting up the procedure to use this method and this would be a desirable technique for D₂O determination when completed.

CALCULATIONS

The dilution volume determined by the D2O procedure actually represents hydrogen volume rather than water volume. Evidence from animal studies suggests that hydrogen space over-estimates water space by 2-6 percent (Wong et al., 1988; Schoeller, 1990). Therefore, hydrogen space should be adjusted by about 04% to obtain the most accurate TBW value. This is most readily accomplished by dividing the D2 dilution space values by 1.04 (Schoeller, 1990).

In general, the equation used is:

$$\text{D2 dilution space} = \frac{(\text{volume D2O ingested} - \text{volume excreted})}{\text{final D2O equilibrium concentration}}$$

When isotope ratio mass spectrometry is used, the assay results are expressed relative to a standard, usually a seawater or precipitation standard such as standard mean ocean water (SMOW) or standard light arctic precipitation (SLAP). The formula utilized for calculating TBW is:

$$\text{D2 dilution space} = (d/MW) * (APE/100) * (18.02 / (R_{std} * \Delta \text{D2O}))$$

Where:

d=dose of D2O

MW=molecular weight

APE- atom percent excess of deuterium

R_{std}= ratio of deuterium to hydrogen in the standard

Delta= increase between baseline and post-ingestion samples (Dempsey et al., 1984).

The formula provided by Halliday and Miller, (1977) is one of the most comprehensive:

$$\text{D2 dilution space (kg)} = (D_{fd} * D_e) / [(p-x) * 1000]$$

Where:

$$D_e = (A - C(y-x)) / D_f$$

Where:

D_f=D₂O concentration of administered D₂O
x=ppm D₂O of baseline fluid sample
p=ppm D₂O of equilibrated fluid sample
A=weight of D₂O dose administered
C=weight of deuterium passed in urine
y=ppm in excreted urine

Weight-volume doses can be converted to volume/volume by correcting for the density of D₂O which is 1.105 at 25°C (Thomson, 1963).

SUMMARY

D₂O dilution techniques offer a safe, well-tested method of determining TBW. Saliva, which may be easily and safely collected, serves as a suitable body fluid sample. Numerous options are available for deuterium assay. JSC laboratories have potential access to GC and isotope-ratio mass spectroscopy, but neither method is currently operational.

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